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The bacteriological quality of minced beef in the U.K.

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SUMMARY

Minced (ground) beef from three supermarkets, three intermediate-sized chain butchers and three small family butchers in each of three geographical areas was examined three times in warm weather and three times in cool. The total viable count (37 and 20°C), numbers of Enterobacteriaceae (37 and 17°C), and presumptive coliforms did not differ significantly between shop type or season. Statistically significant differences in numbers of faecal Streptococci, *Staphylococcus aureus* and *Clostridium perfringens* were too small to be of commercial importance, or diagnostic value.

INTRODUCTION

The possible imposition of microbiological standards has stimulated several surveys of the bacteriological quality of minced (ground) beef in the U.S.A. and Canada (Law, Yang & Mullins, 1971; Duitschaever, Arnott & Bullock, 1973; Al-delaimey & Stiles, 1975; Berry & Chen, 1976; Shoup & Oblinger, 1976; Goepfert, 1976; Pivnick, *et al.* 1976; Duitschaever, Bullock & Arnott, 1977; Foster, Fowler & Ladiges, 1977). Surveys have also been carried out in Tehran (Karim, 1977) and Turkey (Sumner, 1978). Guidelines or standards have been suggested, although both Goepfert & Kim (1975) and Westhoff & Feldstein (1976) have questioned the utility of standards or guidelines in the protection of public health, from bacteriological survey data and the behaviour of selected food-borne pathogens in raw ground beef.

The present study was undertaken to determine the bacteriological quality of minced beef at retail sale in the U.K., the variability of that quality and the value of different bacteriological indices in assessing it. This survey sampled nine shops (three supermarkets, three intermediate-sized chain butchers and three small family butchers), in each of three geographical areas. Each shop was sampled three times in warm weather and three times in cool, to give a total of 162 samples, the temperatures being measured within a few minutes of purchase. Each sample was subjected to eight bacteriological tests and the pH and the gross chemical analysis were measured.

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MATERIALS AND METHODS

Sample

Approximately 350 g of minced beef was purchased between 11.00 a.m. and 12.00 a.m. and its temperature was recorded within 5 min. The sample was then transported to the laboratory on ice packs in an insulated container and held at 1°C until analysed. All samples were analysed bacteriologically within 4 h of purchase and then frozen for fat analysis.

Bacteriological analysis

Twenty-five grams of mince were added to 225 ml of maintenance medium (MM) comprising (% w/v): Bacteriological peptone (Oxoid L37), 0·1; sodium chloride, 0·85; pH 7·0; in an Atomix blender (M.S.E. London, SW1) and macerated at slow speed for 30 s and high speed for 60 s. The following counts were made by spreading duplicate 1/50 ml drops or 0·5 ml samples of decimal dilutions on the media indicated.

Total viable count (*TVC*)

Plate count agar (PCA) (Oxoid CM 325) + 1 % NaCl incubated at 37° for 24 h and 20° for 96 h.

Calculation of viable counts

Results were calculated using counts at two or more dilutions with weighting for dilution, as described by Farmiloe *et al.* (1954).

Presumptive coliform (*PC*)

MacConkey's bile lactose agar without salt (BLA) (Oxoid CM 76) incubated at 37°C for 24 h. From each BLA plate, up to ten typical coliform colonies (large, dark pink and non-mucoid) were subcultured: those giving positive Eijkman test at 44°C and a positive indole reaction at 44°C were deemed to be *E. coli* type I.

Enterobacteriaceae (*ENT*)

Violet red bile agar (VRBA) (Oxoid CM 107) + 1 % glucose overpoured with VRBA incubated at 37°C for 24 h and 17°C for 96 h. Dark red colonies, 1–2 mm diam. surrounded by a reddish zone were counted.

Faecal streptococci (*FS*)

Kanamycin aesculin azide agar (KAAA) (Oxoid CM 481), incubated at 37°C for 24 h. Colonies surrounded by black haloes were counted.

Staphylococcus aureus (*SA*)

Baird-Parker's medium (BP) comprising (% w/v); tryptone (Oxoid L42), 1·0; lab-lemco beef-extract (Oxoid L29), 0·5; yeast extract (Oxoid L21), 0·1; sodium pyruvate, 1·0; glycine, 1·2; lithium chloride, 0·5; Davis' Standard New Zealand Agar, 2·0; plus 5 ml egg yolk emulsion (Oxoid SR47) and 0·3 ml of 3·5 %

potassium tellurite solution; incubated at 37°C for 24 h. Colonies which were black and shiny with a narrow white entire margin surrounded by a zone of clearing were recorded as presumptive *Staph. aureus*.

Clostridium perfringens (*CP*)

Two millilitres of macerate were added to each of five 10 ml amounts of molten egg-yolk-free tryptose-sulphite-cycloserine (TSC) medium (Hauschild & Hilsheimer, 1974) at 45°C in Petri dishes and mixed. After the medium had solidified it was overlayed with the same medium previously cooled to 45°C and incubated anaerobically at 40–43°C for 18 h (in BTL anaerobe jars, under hydrogen containing 'Deoxo' room temperature catalyst.)

From each group of five plates ten representative colonies were sub-cultured in 20 ml amounts of TPYG + cycloserine comprising (% w/v): trypticase (BBL), 2; Bacto-peptone (Difco), 0·5; yeast extract (Oxoid), 0·2; glucose, 0·4; cycloserine, 0·04; pH 7·0. Glucose and cycloserine were added just before inoculation, the glucose having been autoclaved separately (115°C/10 min) as a 20% (w/v) solution and the cycloserine filter-sterilized (Millipore, 0·22 μm) as a 1% solution. After incubation for 18 h at 40–43°C the resultant growth was plated on blood agar (BA) prepared by incorporating 2–4% of Davis' Standard New Zealand Agar into Hartley's digest broth, and adding 5% of oxalated horse blood (Wellcome Biological Reagents, Beckenham, Kent) to the molten agar (45–50°C) before pouring plates. Colonies were then plated on lactose–egg yolk medium (Willis & Hobbs, 1959) and incubated for 18 h at 40–43°C.

Salmonella

Ten grams of mince were added to 100 ml selenite broth base (Oxoid CM 395) with 1% mannite and 2 ml of sodium hydrogen selenite (20% solution in sterile distilled water). Growth at 37°C was subcultured at 24 and 48 h onto phenol red brilliant green agar (Oxoid CM 329).

Temperature

The air temperature outside the shop and the temperature of the mince were measured immediately after purchase using a Digitherm, digital readout thermometer (Kane-May, Burrowfields, Welwyn Garden City).

pH

The pH of the macerate was measured using a Model 29 pH meter (Radiometer, Copenhagen).

Fat

Samples of the minced beef were freeze-dried and extracted in a Soxhlet apparatus with 40–60°C petroleum spirit for 8 h and the extracted fat weighed. The fat-free dry weight was obtained by drying the defatted sample to constant weight in a vacuum oven at 70°C. Moisture content was estimated by difference.

Table 1. *Minimum, maximum and mean values for eight bacterial counts and concomitant data on minced beef*

	Minimum	Maximum	Mean
Total viable count (TVC) 37°C	4·10	7·62	6·11*
Total viable count (TVC) 20°C	5·51	9·00	7·37*
Enterobacteriaceae (ENT) 37°C	<1·00	6·51	4·31*
Enterobacteriaceae (ENT) 17°C	<1·00	6·60	4·61*
Faecal streptococci (FS)	<1·00	4·07	2·46*
Staph. aureus (SA)	<0·70	3·58	0·50*
Cl. perfringens (CP)	<0·00	3·46	0·63*
Presumptive coliforms (PC)	<1·00	5·11	2·55*
Fat (%)	5·95	36·47	22·50
Temperature (°C) at purchase	−2·50	17·50	5·20
Ambient temperature (°C)	8·50	22·50	15·27
pH of macerate	5·65	6·60	5·91

* Log_{10} number of organisms per gram.

Table 2. *The effect of shop type and season on bacterial numbers (log_{10} per g) in minced beef*

	Small		Intermediate size		Supermarket	
	May/Nov.	June/July	May/Nov.	June/July	May/Nov.	June/July
TVC 37*	6·058	6·424	6·038	6·236	5·939	5·979
TVC 20	7·190	7·631	7·230	7·292	7·502	7·348
ENT 37	4·081	4·400	4·306	4·480	4·498	4·121
ENT 17	4·456	4·783	4·441	4·836	4·686	4·468
FS	$2·457_a$	$2·961_b$	$2·284_a$	$2·571_{ab}$	$2·132_a$	$2·364_a$
SA	$*0·786_b*$	$*0·131_a*$	$0·715_b$	$0·534_a$	$0·548_a$	$0·304_a$
CP	$0·439_a$	$0·539_a$	$0·838_b$	$0·707_b$	$*0·918_b*$	$*0·313_a*$
PC†	2·610	2·681	2·360	2·851	2·493	2·280

Within a row means with different subscripts are significantly different ($P < 0·05$). Least significant difference between any two means = 0·49.

* See Materials and Methods.

† *ca.* 53 % of isolates tested were *E. coli* I.

RESULTS

The minimum, maximum and mean values for eight different bacterial indices (log_{10} per g), fat (%), temperature (°C) of mince at purchase, ambient temperature, and pH value of mince are given in Table 1. A three-way analysis of variance of these data, comparing shop type (small, intermediate-sized, supermarket), season (May or Nov.: cool or June/July: warm) and bacteriological count showed that numbers of the different types of bacteria differed significantly, but that there was no significant effect due to season or shop type. The variability of the different bacterial indices was of the same order, the difference between the highest and lowest numbers recorded being of the order $\times 10^3$ to $\times 10^4$ (3–4 in Table 1). A small, but significant interaction ($P < 0·05$) between shop type and season seemed to be due to counts of CP and SA (italics in Table 2).

The three-way table of means (Table 2) illustrates the absence of any significant differences in five of the bacterial counts TVC 37°C, TVC 20°C, ENT 37°C, ENT 17°C and PC. In small and intermediate shops, numbers of FS were significantly higher in the warmer season than the cool and numbers of SA were higher in the cool season than the warm. In intermediate shops and in supermarkets numbers of CP were significantly higher in the cool season than in the warm. Although these counts of FS, SA and CP were significantly different, the differences in numbers would be of no commercial importance or diagnostic value.

There was no correlation between TVC 37 or TVC 20 and fat or temperature at purchase. The pH was significantly and positively correlated with counts, i.e. there was a trend to higher bacterial counts with increasing pH value. Using the resultant line it was possible to remove the variability in the counts associated with pH and to re-analyse the data. This marginally decreased the residual (i.e. unexplained) variability (the least significant difference (LSD) between any two means was reduced from 0·49 to 0·48), but did not materially alter the mean values or the conclusions drawn from Tables 1 and 2.

DISCUSSION

In the U.K. minced beef is usually either minced in bulk for sale on a given day or a small amount is minced fresh on demand. The bacteriological count of minced beef is partly a reflexion of the status of the meat used (Chestnut *et al.* 1977) and if mince is sold fairly rapidly it is not normally of great consequence. Relatively low numbers of some bacteria of public health significance, e.g. *Cl. perfringens* and *Staph. aureus*, are found in mince but provided it is well refrigerated their growth is minimal (Goepfert & Kim, 1975). Problems may arise if mince is not refrigerated and particularly if it has been stored inadequately and is then cooked insufficiently before consumption.

The bacteriological tests used in this survey whilst not identical with those of other surveys were sufficiently similar to provide comparable data.

The analysis of our U.K. data showed that the numbers of bacteria in minced beef at retail were essentially unaffected by the type of shop and did not differ significantly in warm and cool weather. There were significant numerical differences between the different counts. Therefore, if a measure of overall microbial quality is needed, TVC 37°C would supply it quickly. Too few samples contained *Salmonella* (2 of 162) to test the relationship between the coliform count and the presence of *Salmonella*. However, there seems little point in making coliform counts as an indicator of the occurrence of *Salmonella* since the only two samples of *Salmonella* had < 10/g and 1000/g coliforms. These findings resemble those of Miskimin *et al.* (1976), who found no relation between total aerobic plate count, *E. coli* or total coliform and the presence of pathogens in raw and ready-to-eat food products.

The present results suggest that the microbiological quality of minced beef in the U.K. is very similar to that recently reported by workers in the U.S.A. and Canada. Even making allowance for slight differences in bacteriological techniques, the number of bacteria in minced beef seems to have changed little in the

past 60 years (Foster *et al.* 1977) and less than might be expected considering the cost and effort of implementing and regulating 'hygiene' in slaughterhouses and retail outlets.

The use of bacteriological standards in an attempt to improve the quality of mince has been suggested in several American States (Wehr, 1978). Most propose using total viable count for overall quality and the *E. coli* or faecal streptococci count as a measure of hygiene. The experiences of Oregon are well documented (Meat Bacterial Standards Review Committee Report of the State of Oregon, 1977) and the cost of enforcing meat bacterial standards were not justified by the benefits derived by the people of the State. It was concluded that there has probably not been a significant change in the quality of ground beef because of the standards, and that there is a need for education of food companies, retailers and consumers in the handling and cooking of meat and meat products to minimize spoilage and food poisoning. Following the publication of the report the Oregon standards have been rescinded and replaced by bacterial criteria which are only advisory and which do not carry criminal liability (Wehr, 1978). Until there is clearer evidence that minced beef is frequently implicated in food-borne illness, there seems little point in applying bacteriological criteria.

Those seeking to compare the bacteriological status of a product with data of others will find, as we did, that statistical comparisons from published scientific papers are impossible because different workers present their data in different ways. It would greatly facilitate comparisons if a standardized format for bacteriological data were developed.

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